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Vertebrate Organogenesis: Getting the Heart into Shape

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Recent mutant analysis in zebrafish points to an important role for oriented cell division in cardiac chamber formation and reveals its molecular control by a novel signal from the heart's interior.

The heart, like many organs, undergoes dramatic changes in its three-dimensional form as the embryo develops and functional demands intensify. Before it becomes a multi-chambered organ, the heart exists as a simple tube made up of myocardium (muscle) lined by endocardium (endothelium) [1] (Figure 1). As this thin-walled tube bulges outward, the chambers emerge and eventually acquire characteristic dimensions of curvature and thickness. How is this specific form achieved? Cell proliferation and increases in cell size are known to contribute to chamber thickening [2], and regional differences in proliferation rate could cause differential chamber growth. Besides the rate of cell growth, however, the pattern of cell growth may also play an important role. Work from Mably, Fishman and colleagues [3], published recently in *Current Biology*, now provides evidence that an additional mechanism —orientation of cell division —underlies acquisition of chamber form. Further, they show that patterned growth of the chambers depends on a novel endocardial signal encoded by the *heart of glass* (*heg*) gene.

If oriented cell growth operates in the heart, how could it contribute to formation of the cardiac chambers? Oriented cell growth could facilitate regional bulging of the heart tube, as it expands in a directed fashion to form chambers, and could contribute to thickening of chamber walls. Support for a role of oriented cell growth in chamber formation comes from recent studies by Meilhac, Buckingham and coworkers [4], who used clonal analysis to examine patterns of oriented cell growth in the embryonic mouse heart. They used a modified *lacZ* reporter gene which is activated in cardiomyocytes at low frequency by intragenic recombination [5], and retrospectively documented the resulting β-galactosidase-positive clones at various stages of heart development. Interestingly, characteristic patterns of clonal organization emerged in specific chambers, directly correlating with characteristic aspects of chamber morphology. In the left ventricle, for example, bulging of the walls was faithfully reflected by extensions of clones oriented perpendicularly to the chamber's long axis. Meilhac *et al.* [4] suggest that orientation of cell division could explain the shapes of clonally related cell groups and, ultimately, drive chamber morphogenesis, a possibility supported by the results of computer simulations.

What might be the result of disrupting oriented cell growth in the developing heart? Through genetic screens in zebrafish, it has been possible to identify specific mutant phenotypes suggestive of defects in chamber growth [6–8]. For example, the *heart of glass* mutation causes a phenotype that could result from disruption of oriented cell division [3]. In contrast to wild-type, the *heg* mutant myocardium never thickens and consists only of a single layer of cells (Figure 1). The *heg* mutant chambers become abnormally expanded and fail to maintain circulation. Although mutant myocardial cells are slightly smaller, the total number of cells is consistent between wild-type and *heg* mutant chambers. The *heg* mutation thus appears primarily to affect the pattern of myocardial growth.

Cloning of *heg* has revealed a molecular signal that directs patterned growth of the myocardium and suggests a new role of the endocardium in this process. The *heg* gene encodes a novel protein with several alternative splice variants [3]. The effects of morpholinos directed against the three splice variants predict that a membrane-associated form of the protein, rather than a secreted form, is the primary effector of *heg* function in the developing heart. Interestingly, expression of *heg* is restricted to the endocardial layer of the heart. Mably *et al.* [3] propose a model whereby normal chamber thickening proceeds via concentrically oriented cell divisions (Figure 1). Heg activity might regulate this growth pattern by signaling from the endocardium through direct interaction with the overlying myocardium.

Is Heg sufficient to direct oriented growth, or does it cooperate with a suite of signals? One clue to potential complexity comes from recent studies of epigenetic influences during chamber formation. It had long been suspected that hemodynamic shear forces play a role in heart development, as endothelial cells in culture respond to alterations in fluid shear stress via changes in gene expression [9]. Novel imaging techniques allowed this question to be addressed *in vivo*, demonstrating high-velocity, high-shear conditions in the zebrafish heart [10]. When blood circulation into or out of the heart was mechanically blocked, cardiac morphogenesis was disrupted, indicating that flow indeed influences form. In particular, valve formation, which requires reciprocal endocardial–myocardial signaling [11–13], was severely affected. This epigenetic phenomenon is probably not heart-specific: changes in hemodynamic flow, which may be transmitted via stretch receptors in the endothelium, also affect glomerulus development in the kidney [14].

Might shear forces sensed by the endocardium have an impact on the endocardial-to-myocardial signaling inferred from analyses of *heg* mutants? This issue could be difficult to resolve in *heg* mutants, as their circulation ceases. It would, however, be interesting to

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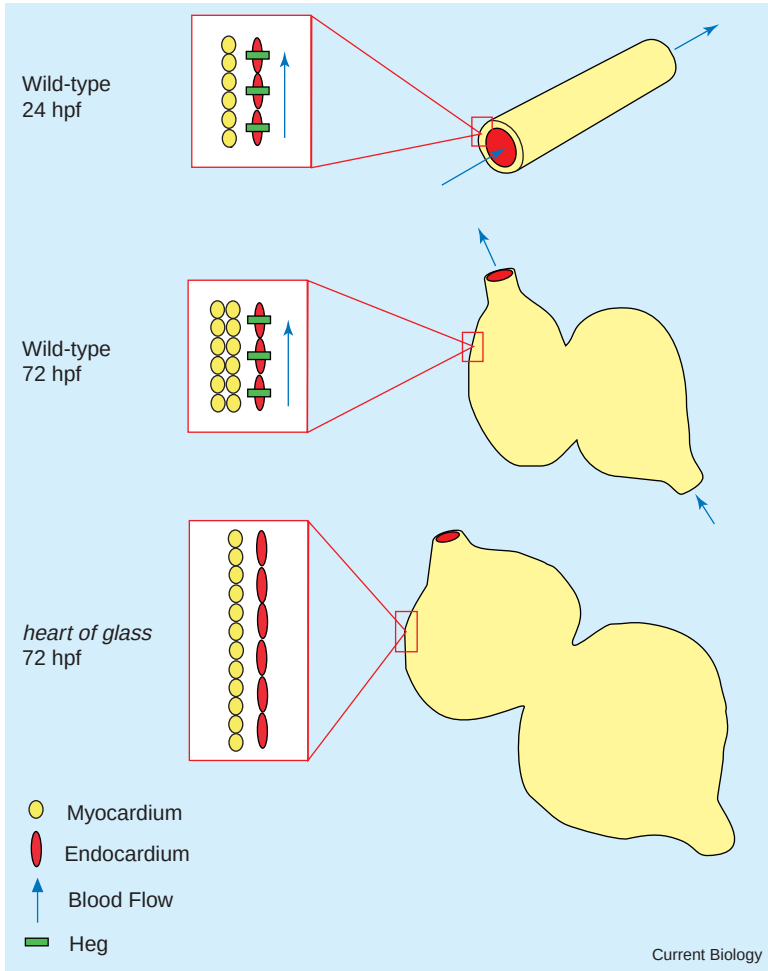

Figure 1. Cardiac chamber formation in zebrafish and a model of Heg function.

The cardiac chambers emerge from a thin-walled tube, at 24 hours post fertilization (hpf), to acquire characteristic dimensions of shape and thickness by 72hpf. In this model, Heg signals from the endocardium to myocardium, resulting in oriented cell division, thickening of chamber walls, and acquisition of normal chamber form. In the absence of Heg function, although cell number is not affected, chamber growth is not properly oriented, resulting in grossly enlarged and thin-walled chambers. The proximity of hemodynamic flow to the endocardium is also indicated.

examine whether *heg* expression is responsive to fluid forces. It will also be important to characterize the function of the *heg* homologue in mouse, where oriented clonal growth patterns are now well-documented with respect to chamber form [4]. As different chambers possess distinct morphologies, it seems likely that oriented cell division is regulated in a region-specific manner. There may be multiple factors participating in regionalized interplay between myocardium, endocardium, and the sensation of shear forces. Fortunately, given the variety of zebrafish mutants featuring abnormally thick or thin cardiac chambers [6–8], the identities of these other regulators may already be awaiting discovery.

References

1.Harvey, R.P. (2002). Patterning the vertebrate heart. Nat. Rev. Genet. *3*, 544-556.

2.Li, F., Wang, X., Capasso, J.M., and Gerdes, A.M. (1996). Rapid transition of cardiac myocytes from hyperplasia to hypertrophy during postnatal development. J. Mol. Cell Cardiol. *28*, 1737-1746.

3.Mably, J.D., Mohideen, M.-A.P.K., Burns, C.G., Chen, J.-N., and Fishman, M.C. (2003). heart of glass regulates the concentric growth of the heart in zebrafish. Curr. Biol. *13*, 2138-2147.

4.Meilhac, S.M., Esner, M., Kerszberg, M., Moss, J.E., and Buckingham, M.E. (2004). Oriented clonal cell growth in the developing mouse myocardium underlies cardiac morphogenesis. J. Cell Biol. *164*, 97-109.

5.Meilhac, S.M., Kelly, R.G., Rocancourt, D., Eloy-Trinquet, S., Nicolas, J.F., and Buckingham, M.E. (2003). A retrospective clonal analysis of the myocardium reveals two phases of clonal growth in the developing mouse heart. Development *130*, 3877-3889.

6.Stainier, D.Y., Fouquet, B., Chen, J.N., Warren, K.S., Weinstein, B.M., Meiler, S.E., Mohideen, M.A., Neuhauss, S.C., Solnica-Krezel, L., Schier, A.F., *et al.* (1996). Mutations affecting the formation and function of the cardiovascular system in the zebrafish embryo. Development *123*, 285-292.

7.Chen, J.N., Haffter, P., Odenthal, J., Vogelsang, E., Brand, M., van Eeden, F.J., Furutani-Seiki, M., Granato, M., Hammerschmidt, M., Heisenberg, C.P., *et al.* (1996). Mutations affecting the cardiovascular system and other internal organs in zebrafish. Development *123*, 293-302.

8.Warren, K.S., Wu, J.C., Pinet, F., and Fishman, M.C. (2000). The genetic basis of cardiac function: dissection by zebrafish (*Danio rerio*) screens. Philos. Trans. R. Soc. Lond. Biol. *355*, 939-944.

9.Topper, J.N., and Gimbrone, M.A., Jr. (1999). Blood flow and vascular gene expression: fluid shear stress as a modulator of endothelial phenotype. Mol. Med. Today *5*, 40-46.

10.Hove, J.R., Koster, R.W., Forouhar, A.S., Acevedo-Bolton, G., Fraser, S.E., and Gharib, M. (2003). Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis. Nature *421*, 172-177.

11.Dor, Y., Camenisch, T.D., Itin, A., Fishman, G.I., McDonald, J.A., Carmeliet, P., and Keshet, E. (2001). A novel role for VEGF in endocardial cushion formation and its potential contribution to congenital heart defects. Development *128*, 1531-1538.

12.Brown, C.B., Boyer, A.S., Runyan, R.B., and Barnett, J.V. (1999). Requirement of type III TGF-beta receptor for endocardial cell transformation in the heart. Science *283*, 2080-2082.

13.Ramsdell, A.F., and Markwald, R.R. (1997). Induction of endocardial cushion tissue in the avian heart is regulated, in part, by TGFbeta-3-mediated autocrine signaling. Dev. Biol. *188*, 64-74.

14.Serluca, F.C., Drummond, I.A., and Fishman, M.C. (2002). Endothelial signaling in kidney morphogenesis: a role for hemodynamic forces. Curr. Biol. *12*, 492-497.